that Glu184 is essential for its catalytic activity. We have also determined the residues involved in substrate recognition by single amino-acid substitution experiments based on the structure. These results indicate that a large conformational change of sub-domain is required to exert the muramidase activity. We will discuss a possible PG-hydrolyzing mechanism of FlgJ in flagellar assembly.

1296-Pos

Structure of the Newcastle Disease Virus F Protein in the Post-Fusion Conformation

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Newcastle disease virus (NDV) is a member of the Paramyxoviridae family. The NDV fusion (F) glycoprotein, which is responsible for merging the viral and cellular bilayers during entry. The X-ray crystal structures have been solved of F proteins in the post-fusion and the pre-fusion conformations, providing atomic level information regarding the conformational transitions accompanying fusion. However, our understanding of the similarities between different F glycoproteins in these two conformational states remains incomplete.

Here, we present the crystal structure of the secreted, uncleaved ectodomain of the NDV F protein. Previous structural analysis of a related NDV F protein was missing key elements of the functional regions of the protein, including two helical segments (HRA and HRB) that assemble into a stable six helix bundle (6HB) in the post-fusion form. We have produced the NDV F protein in pre- and post-fusion conformations, using analogous constructs that produced a pre-fusion PIV5 F structure and a post-fusion HPIV3 F structure. We demonstrate that the two NDV F proteins exhibit the pre-and post-fusion forms through EM analysis and we have solved the crystal structure of the post-fusion form of the NDV F protein. In contrast to the previously determined NDV F structure, our new crystal structure contains the 6HB at the base of the stalk region, consistent with the EM observations and the previously determined HPIV3 F structure. Global superposition of the NDV and HPIV3 structures demonstrates maximum correspondence between distal portions of the structures, with orientation or adjustments in linking domains and the extended HRA stalk. Electrostatic profiles of the NDV, HPIV3, and PIV5 F structures show elements of conserved charge distributions despite significant sequence differences in these glycoproteins, which may be important for their common functionality.

1297-Pos

Structure of DNA Binding Domain of Plant Telomere Binding Proteins Represents Unique Features of Telomere Binding Protein Family

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Telomeres, the ends of linear eukaryotic chromosomes, are composed of short repeats of G-rich sequences and play essential roles in genome stability with various telomere binding proteins. To characterize the binding mode of plant telomere DNA and telomere binding protein, we determined the structures of DNA binding domain and telomere complex of NgTRF1, atTRF and RTBP1, double strand telomere binding proteins of plants, by multidimensional NMR spectroscopy and X-ray crystallography. We have identified the DNA binding interface of the DNA binding domain of TBPs, which is composed of 4 α-helices by means of chemical shift perturbation analysis. The complex crystal structure of $NgTRF1^{561\ 681}$ and plant telomere DNA $(TTTAGGG)_2$ have shown the molecular details of the interaction between them and we confirmed the interaction biochemically through site-directed mutagenesis. From the comparison with the structure of human telomere binding protein, we tried to show the unique features of plant telomere binding protein in the mode of telomere DNA binding as well as the similarity with the telomere binding proteins in other organisms. To our knowledge, this is the first report of the complex structure of telomere binding protein and telomere DNA in plant.

1298-Pos

Structural and Functional Characterization of an Unusual SH3 Domain from the Fungal Adaptor Protein Bem1

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Protein interactions form the basis of most biological processes and in eukaryotes are often mediated by conserved modular domains that recognize linear motifs. Among the most common protein interaction domains is the SH3 domain that generally recognizes PxxP containing peptides. SH3 domains are approximately 60 amino acids long and are composed of five beta strands. We are studying the SH3 domain from the fungal adaptor protein Bem1p that plays an important role during polarized growth and activation of MAPK signaling pathways. This SH3 domain is unusual, even though its sequence conforms to the SH3 domain consensus, it requires an extra 40 amino acids at its C-terminus for folding. Furthermore, in addition to binding PxxP containing peptides, it also binds the Cdc42p GTPase in a PxxP-independent manner. We are using in vitro binding assays and NMR spectroscopy to structurally and functionally characterize this unusual SH3 domain. Contrary to a previous report, we find that the Bem1 SH3 domain can simultaneously bind the Cdc42p GTpase and PxxP-containing peptides and that the binding of one does not affect the affinity for the other. Structural characterization by NMR shows that the extra sequence contains two alpha helices that pack tightly against the SH3 domain and thus form an integral part of the fold. Our findings provide with an example of how a common protein interaction domain can evolve to have additional atypical structural features and associated functions.

1299-Pos

Structural Investigation of a Fibronectin Type III Domain Tandem from the A-band of the Titin

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Single molecules of the giant muscle protein titin span half muscle sarcomeres, from the Z-disk to the M-band, and have key roles in sarcomere assembly and elasticity. In the A-band titin is attached to thick filaments and here the sequence shows fibronectin type III and immunoglobulin-like domains. These are mostly arranged in regular patterns of eleven domains called the large super-repeats. The large super-repeat occurs eleven times and this entire region thus forms nearly half of the titin molecule. Through interactions with myosin and C-protein, it is involved in thick filament assembly. We are determining the atomic structure, dynamical properties and the inter-domain arrangement of overlapping double and triple domain fragments of the large super-repeat by NMR spectroscopy. Ultimately, we hope to combine the data to reconstruct the overall conformation of the super-repeat. Here we investigated the A59-A60 domain tandem, which was expressed in bacteria from cDNA. The assignment of the backbone atoms was obtained using triple resonance NMR experiments. An initial structure was determined by backbone chemical shifts and homology modeling using the CS23D and Rosetta software packages. It was refined using RDC data to give realistic models for both domains. As we expected, these are both double-β-sheet sandwich structures characteristic of fibronectin type III domains. We are also carrying out relaxation measurements to probe the dynamics of the domains and their linker region.

1300-Pos

Localization of the Fission Yeast U5.U2/U6 Spliceosome Subunits

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The spliceosome is a dynamic macromolecular machine that catalyzes the excision of introns from pre-mRNA to generate protein-coding transcripts. The megadalton-sized spliceosome is composed of four small nuclear RNPs (U1, U2, U5, and U4/U6) and numerous pre-mRNA splicing factors. The formation of an active spliceosome is hypothesized to occur in a stepwise manner requiring the assembly and disassembly of large multiprotein/RNA complexes. A promising structural approach to obtain information about spliceosome complexes is single-particle cryo-electron microscopy (cryo-EM), a powerful technique that is ideal for determining the structures of large dynamic complexes at protein concentrations too low for crystallization. Formerly, our group determined structure of the fission yeast U5.U2/U6 spliceosome complex by cryo-EM. This U5.U2/U6 spliceosome complex contains the U2, U5, and U6 snRNAs, pre-mRNA splicing intermediates, U2 and U5 snRNP proteins, the Nineteen Complex (NTC), and second-step splicing factors. However, the location of these subunits in the complex was not determined. Using antibody labeling and single particle EM we are now localizing these individual subunits within the density map of the U5.U2/U6 spliceosome complex. This work now enables us to propose a structural model for U5.U2/U6 organization.

1301-Pos

3D Solution Structure of the C-terminal Chromodomain of the Chloroplast Signal Recognition Particle

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Chloroplasts use chloroplast signal recognition particle (cpSRP) pathway to import important cargo like light harvesting chlorophyll protein (LHCP).